第6卷 第4期 动 物 学 研 究 Vol. 6, No. 4
1985年11月 ZOOLOGICAL RESEARCH Nov., 1985

On the nucleoli of the dinoflagellate *Prorocentrum*

Li Jingyan

(Kunming Institute of Zoology, Academia Sinica)

Abstract

To date no nucleolus had been observed in *Prorocentrum* under the light microscope. The author failed to show the nucleoli of *P. micans* and *P. cassubica* with eosin in 70% alc or with methyl green-pyronin. But when these dinoflagellates were treated with an Ag-1 technique which had been improved for demonstrating NORs in unicellular organisms, nucleoli were stained dark brown or black, while all other parts showed no colour. When the materials were stained well, only the central part of the nucleolus was stained. Under the electron microscope, it was observed that all the silver grains were concentrated in the pars fibrosa of the nucleolus. *P. cassubica* had only one small oblate nucleolus attached to the nuclear envelope, with NOR usually in the shape of the letters O or C. *P. micans* had 1—7 nucleoli of various sizes and shapes with NORs in various complicated forms. The number of nucleoli bore a certain relationship to the living state of the dinoflagellate. One day after fresh medium was added, cells with 3 nucleoli were most common, and 28.5% of the individuals had 4—6 nucleoli. Cells having only one nucleolus accounted for 8.6%. 3 days after, cells with 2 nucleoli became dominant, and those with 4—6 decreased to 18.4%. After a month, cells with 1 nucleolus became most abundant, cells having 4 nucleoli decreased to 2.4%, and no cells had 5 or 6 nucleoli.

Key words: Nucleolus dinoflagellate silver-staining culture

Introduction

It has been reported that no nucleolus occurs in several dinoflagellates, especially in species belonging to *Prorocentrum* and *Exuviella* (Dodge, 1966).

This is surprising. Although nucleoli were observed afterwards in *Prorocentrum micans* under electron microscope (Zingmark, 1970), it remains difficult to get a complete picture of nucleoli for study by ultrathin sections. To investigate the variation in nucleolus number, the present author improved the Ag-1 procedure for demonstrating nucleolar organizer regions(NORs) in unicellular organisms under both light and electron microscopes (Li Jing-yan, 1981). In the present work, the author used this improved technique to demonstrate nucleoli in *Prorocentrum micans* and in *P. cassubica*. For the first time, the morphology of these nucleoli could thus be studied under microscope and their numbers counted. *Prorocentrum micans* is an organism which may cause red tides. The investigation of their nucleoli may be of help to predict these.

Materials and methods

Prorocentrum micans (LB1136) obtained from the Culture Centre of Algae and Protozoa (Cambridge, England) and *P. cassubica* (LB1596) obtained from The Culture Collection of Algae at the University of Texas were grown in AE_{60} medium at 20°C.

Absolute alcohol, 10% formalin, Carnoy's fluid (3 : 1), and 2% glutaraldehyde (phosphate buffer, pH 7.4) were used as fixatives. Part of the fixed material, after washing, was used for preparing smears. Three staining methods were used: 1. 0.5% cosin in 70% alcohol, 2. Unna's methyl green -pyronin, 3. the improved Ag-1 technique (Li Jing-yan, 1981).

All the steps in the improved Ag-1 technique were carried out in centrifuge tubes. Material fixed with alcohol, formalin, or glutaraldehyde should be treated with Carnoy's fluid (3 : 1) for 5 to 10 min. For microscopic observation, after thorough washing with redistilled water, materials were stained with freshly prepared 50% $AgNO_3$ containing 1% acetic acid, at 37°C for 6 h. For electron microscope observation, staining for 2 or 3 hours proved sufficient. The silver stained materials were washed thoroughly with redistilled water and were then smeared or embedded in Epon 812

Because of the high specificity of this improved Ag—1 procedure, only nucleoli were stained, and all other cell components, including the condensed dinoflagellate chromosomes, had no colour at all. So, the contour of nuclei could not be distinguished. Several smears were counterstained with methyl green to show chromosomes and the edge of nuclei. Part of the fixed material, after washing, was immersed into 0.2% boric acid solution for a few minutes before silver staining. This boric acid treatment would make the condensed

chromosomes pale yellow after silver staining, while the nucleoli were still stained dark.

The materials embedded in Epon 812 were ultrathin-sectioned and stained with uranium acetate and lead citrate.

Observations and discussion

In common histological preparations, cytoplasm and nucleoli in various cells and tissues are always stained rose-pink by eosin alcoholic solution. But when *P. micans* and *P. cassubica* were stained with eosin solution for a long time, there were no nucleolus-like structures demonstrated in nuclei, while cytoplasm was stained rose-pink clearly. The condensed dinoflgellate chromosomes were not stained either.

It could be inferred that the RNA-rich nucleoli would be stained bright red while chromosomes blue-green in the preparations stained with methyl green-pyronin. But this was inconsistent with the cases of *P. micans* and *P. cassubica*. The fact was that the cytoplasm was stained red and chromosomes blue-green, while there were no structures stained bright red in the nuclei of *Prorocentrum*. When the preparations were carefully examined under oil-immersion, sometimes a few small areas stained pale red or pale purple were seen in the nuclei. It was likely that these areas were nucleoli but they were too pale and indistinct to be distinguished from the pale red nucleoplasm around the chromosomes, and only on very few occasions could they be identified as nucleoli. So, it was impossible to investigate the morphology of these dinoflagellates' nucleoli and count their numbers with this staining method.

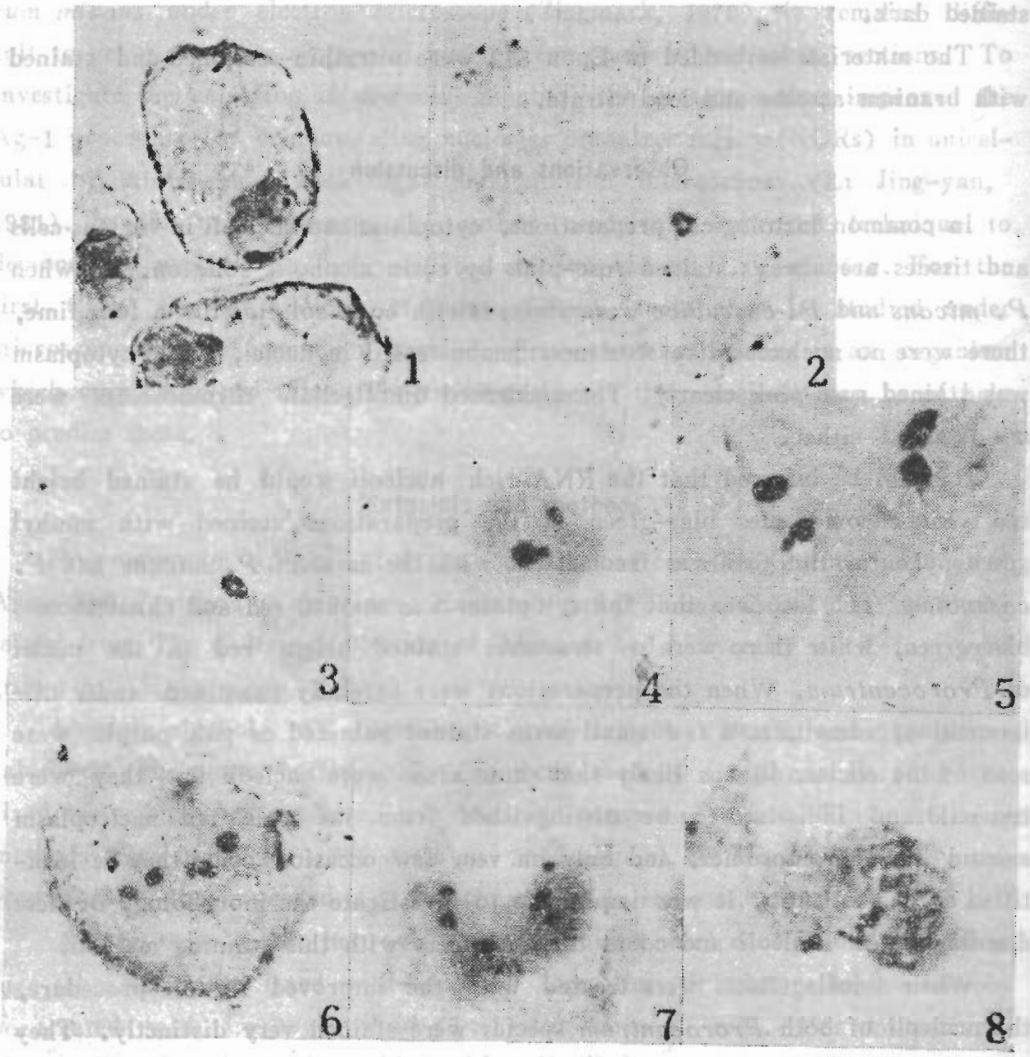
When dinoflagellates were treated with the improved Ag—1 procedure, the nucleoli of both *Prorocentrum* species were stained very distinctly. They were dark brown or deep black, while all the other components in the cell showed no colour at all.

If the cells were not over-stained and the preparations carefully examined, it could be seen that only the central part of the nucleolus was stained and the peripheral region remained colourless (when the cells were over-stained the whole nucleolus was stained) .

Under the eletron microscope (the cells were stained for only 2 or 3 h), it was observed that all the siver grains were concentrated in the central pars fibrosa, and there were no silver grains in the peripheral pars granulosa nor in the condensed chromatin cords within the dinoflagellate nucleolus.

It is known that the silver staining demonstrating NORs may possess very high specificity and there is only one species of protein shown by this silver

[illegible] silver grains were concentrated in the central pars



Explanation of plate

Dinoflagellates stained with the improved Ag—1 technique. Materials had been treated with boric acid before staining with $AgNO_3$ in fig. 1. Materials were counterstained with methyl green in fig. 4, 7 and 8.

Fig. 1—3. *Prorocentrum cassubica*.
Fig. 1. Nucleolus attached to the nuclear envelope.
Fig. 2. and 3. O-shaped NOR.
Fig. 4—8. *Prorocentrum micans*.
Fig. 4. The individual with 2 nucleoli.
Fig. 5. The individual with 4 nucleoli.
Fig. 6. The individual with 5 nucleoli.
Fig. 7. The individual with 7 nucleoli.
Fig. 8. The NOR like a spiral line.

staining. This is an acidic protein participating in the transcription activity on pre-rRNA genes (Hubell *et al.*, 1979). So, the active NOR in dinoflagellate nucleolus is just the pars fibrosa.

The nucleus of *P. cassubica* possesses only one small nucleolus which is oblate and attached to the nuclear envelope. In rare occasions, the nucleolus was located within a nuclear bud extruding into the cytoplasm. This would give the false impression that the nucleolus had departed from the nucleus. It was observed that the NOR in the nucleolus of *P. cassubica* was usually ringlike or C-shaped.

The morphology of the nucleoli of *P. micans* is greatly different from that of *P. cassubica*. Its number of nucleoli in the nucleus is variable. In different individuals, and even in the same nucleus, these nucleoli may vary in size and shape. This is in striking contrast with the nucleolus in *P. cassubica*. Very big nucleoli could be found be found only in nuclei which had one or two uucleoli. The total voume of the nucleoli in one nucleus is not a constant, but varies strongly.

The NORs of *P. micans* take various shapes. They usually look like complicated wound cords, but sometimes they form hollow spheres with thick walls. Occasionally they even appear as long spiral lines in rod-like nucleoli.

Most of the individuals possess 1 to 6 nucleoli, and in very rare cases there are 7. It seems that there is a certain relationship between the number of nucleoli in a nucleus and the physiological state of the dinoflagellate. One day after fresh culture medium was added to the old medium (with about equal volumes), the individuals having 3 nucleoli were the most common (33.3% of all individuals); 28.5% of the individuals had 4 to 6 nucleoli; the individuals with only one nucleolus were at 8.6%. Three days after fresh medium was added, individuals with 2 nucleoli became most numerous (38.8%), cells with 4 to 6 nucleoli decreased to 18.4%, and individuals possessing only 1 nucleolus increased to 13.9%. One month after, the elongate nuclei became spherical, the individuals possessing 1 nucleolus were the most common (36.6%), the

Table 1 The variation of nucleolus number in nucleus of *Prorocentrum micans* with the ageing of culture medium.

Days after adding fresh medium	Individuals with different number of nucleoli				
	1	2	3	4	5—6
1 day	8.6%	28.6%	33.3%	21.4%	7.1%
3 days	13.9%	38.8%	28.9%	14.5%	3.9%
30days	36.6%	31.5%	29.5%	2.4%	0 %

cells with 4 nucleoli were only at 2.4%, and there were no individuals with 5 or 6 nucleoli (Table. 1).

There are two hypotheses to explain why the number of the nucleoli decreases with increasing age of the culture medium. One is that two or more nucleoli fuse into a larger one. But this seems to be inconsistent with the facts, for the individuals living in the aged culture medium usually possess small nucleoli only, and sometimes very small ones. Another explanation is that the decrease is caused by a reduction in protein synthesis within the cells when the conditions become worse. When the biosynthesis of protein decreases, the requirement for the newly formed ribosomal subunits decreases too. As a result more and more nucleolar organizers become inactive and take no part in the construction of the nucleolus.

P. micans is one of the dinoflagellates which may cause red tides. The nucleoli number in these dinoflagellates' nuclei may reflect, at least to a certain extent, their condition. Perhaps examining the nucleoli of these dinoflagellates in the sea might contribute to forecasting red tides.

Acknowledgements

The author expresses his gratitude to Prof. Zhuang Xiao-hui, the director of Shanghai Inst. of Cell Biol., where this work was perfomed. The author is grateful for the assistance of Mr. Qiao Yi-jong and Mrs. Shang Wei-ming.

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*Prorocentrum*属涡鞭毛虫核仁的观察*

李靖炎

(中国科学院昆明动物研究所)

迄今未能在光学显微镜下观察到*Prerocentrum*属的涡鞭毛虫有核仁。本文作者用伊红的酒精溶液和用甲基绿—派若宁法染色，也未能在*Prerocentrum micans*和*Prorocentrum cassubica*的细胞核中显示出核仁来。但是在用专门为显示单细胞生物的核仁组织者区(NOR)而改进了的Ag—1法进行染色时，这两种涡鞭毛虫的核仁都会被染作鲜明的深褐色或深黑色，而身体的所有其他部份，包括染色体，全都不着色。染色适当时可以看出，实际上只是核仁的中央部分被染上色。在电镜下可见，此时所有的银粒全部是集中在核仁的纤维区中。染色的结果表明，*Prorocentrum cassubica*只有一个扁圆形的小核仁，后者是贴附在核膜上，其NOR通常是作O形或C形。与*P. cassubica*不同，*P. micans*的核仁的数量变化很大，可以有一个至七个；其核仁的大小与形状同样也变化很大；其NOR的形状也复杂多变。发现*P. micans*的核仁数量与个体的生活状况有一定的关联：向老的培养液中加入等量的新的培养液一天以后，具有三个核仁的个体是最多的(占三分之一)，具有4—6个核仁的个体占28.5%，只有一个核仁的个体只占8.6%；加入新培养液三天后，具两个核仁的个体变成是最多的(占38.8%)，具4—6个核仁的个体降为占18.4%；加入新培养液一个月以后，只有一个核仁的个体是最多的(占36.6%)，具四个核仁的个体仅占2.4%，没有任何个体有五个或六个核仁。

关键词 核仁 涡鞭毛虫 银染 培养

* 本文曾于1983年在青岛第十一届国际海藻会议上宣读。

本文1985年1月7日收到，1985年7月24日收到修改稿。